Denitrification in Wood Chip Bioreactors at Different Water Flows

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Subsurface drainage in agricultural watersheds exports a large quantity of nitrate-nitrogen (NO_3–N) and concentrations frequently exceed 10 mg L^{-1}. A laboratory column study was conducted to investigate the ability of a wood chip bioreactor to promote denitrification under mean water flow rates of 2.9, 6.6, 8.7 and 13.6 cm d^{-1} which are representative of flows entering subsurface drainage tiles. Columns were packed with wood chips and inoculated with a small amount of oxidized till and incubated at 10°C. Silicone sampling cells at the effluent ports were used for N_2O sampling. ^{15}Nitrate was added to dosing water at 50 mg L^{-1} and effluent was collected and analyzed for NO_3–N, NH_4–N, and dissolved organic carbon. Mean NO_3–N concentrations in the effluent were 0.0, 18.5, 24.2, and 35.3 mg L^{-1} for the flow rates 2.9, 6.6, 8.7, and 13.6 cm d^{-1}, respectively, which correspond to 100, 64, 52, and 30% efficiency of removal. The NO_3–N removal rates per gram of wood increased with increasing flow rates. Denitrification was found to be the dominant NO_3–N removal mechanism as immobilization of $^{15}NO_3$–N was negligible compared with the quantity of $^{15}NO_3$–N removed. Nitrous oxide production from the columns ranged from 0.003 to 0.028% of the N denitrified, indicating that complete denitrification generally occurred. Based on these observations, wood chip bioreactors may be successful at removing significant quantities of NO_3–N, and reducing NO_3–N concentration from water moving to subsurface drainage at flow rates observed in central Iowa subsoil.

SUBSURFACE-DRAINAGE (tile) waters deliver significant quantities of NO_3 to drainage ditches, streams, and rivers of the Midwest (Baker et al., 1975; Gast et al., 1978, Jaynes et al., 1999). Export of N from the Midwest via rivers is a contributing factor to the hypoxia problem in the Gulf of Mexico (Rabalais et al., 1996; EPA Science Advisory Board, 2007). Concentrations of NO_3–N exiting subsurface drains frequently exceed the EPA MCL of 10 mg L^{-1} at all times of the year except late summer and early fall and a substantial fraction of base flow in rivers in tile-drained areas is derived from subsurface drainage discharge (Jaynes et al., 1999; Schilling, 2005). Several communities in Iowa use rivers for their drinking water supply and to remove NO_3 from the river water Des Moines, IA operates the world's largest NO_3 removal system at a cost of $3,000 per day of operation. Research has shown that reduced fertilizer applications alone will not reduce NO_3–N concentrations below 10 mg L^{-1} for a corn–soybean [*Zea mays* L.–*Glycine max* (L.) Merr.] rotation (Baker et al., 1975; Gast et al., 1978; Jaynes et al., 2001).

One strategy for reducing NO_3 exports via agricultural drainage waters is edge-of-field bioreactors or denitrification walls (Jaynes et al., 2008). Denitrification capacity generally decreases with soil depth reflecting the decreased microbial biomass, C substrate, or other electron donors to support denitrification (Parkin and Meisinger, 1989; Yeomans et al., 1992; Sotomayor and Rice, 1996; Richards and Webster, 1999). Consequently, once NO_3 leaches out of the surface soil it is available for leaching to subsurface drains. Bioreactors and denitrification walls are designed to intercept drainage water and enhance denitrification with a solid phase C substrate.

Bioreactors constructed by Blowes et al. (1994) to remove NO_3 from subsurface agricultural drainage water via denitrification were effective in reducing concentrations of 3 to 6 mg NO_3–N L^{-1} in the drain water to <0.2 mg L^{-1} at flow rates of 10 to 60 L d^{-1}. Based on average flow rates of 26 to 29 L d^{-1}, a 2.4 to 10.4 d hydraulic residence time (HRT) can be estimated. Tree bark, wood chips, and leaf compost served as organic C sources to promote NO_3 removal. Concurrent with NO_3 removal, increases in effluent alkalinity and concentrations of Mn (II), and Fe (II), and decreased SO_4^- concentrations were observed. Schipper and Vojvodic-Vukovic (1998, 2000, 2001) effectively removed NO_3 from agricultural ground water by filling a trench with a mixture of soil and sawdust (a denitrification wall) to promote denitrification as water passed through the wall. Over 5 yr 95% of the NO_3–N in upgradient groundwater

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Abbreviations: DOC, dissolved organic carbon; DW, dry weight; HRT, hydraulic residence time.

(5–15 mg NO_3–N L^{-1}) was removed. Removal was attributed to denitrification based on enhanced denitrifying enzyme activity (DEA) within the wall. Declining total N concentrations in the soil/sawdust mix suggested that N immobilization was not a predominating removal process. Jaynes et al. (2008) used a similar wood chip-based denitrification wall in Iowa to remove NO_3 from corn–soybean rotation drainage water. Nitrate concentrations in subsurface drainage water averaged 22 mg NO_3–N L^{-1} in the control and 8.8 mg NO_3–N L^{-1} in the drainage water after passage through the denitrification wall.

Carbon-amended bioreactors and denitrification walls have also been successfully used to treat septic tank field effluent (Robertson et al., 2000; Robertson and Cherry, 1995) and aquaculture waste water (Saliling et al., 2007). Soluble C substrates have also been used to amend groundwater to enhance denitrification (Smith et al., 2001; Hunter et al., 1997; Khan and Spalding, 2004).

Despite the successes of these previous studies in removing NO_3 from subsurface drainage water and NO_3 contaminated shallow groundwater several issues are unresolved. Robertson and Cherry (1995) observed that NO_3 removal was inversely related to groundwater velocity. Nitrate loading into these remediation systems are governed by both flow and NO_3–N concentration. We estimated groundwater flow velocities between 2 and 25 cm d^{-1} to subsurface drains from field measurements in Iowa (Jaynes et al., 1999). Nitrate removal in these systems is governed by the rate of denitrification and the hydraulic residence time within the bioreactor.

Also critical are the roles of N immobilization and N_2O emissions in the NO_3 removal process. These processes have not been specifically quantified and could be significant in systems receiving large quantities of N. Currently, the Intergovernmental Panel on Climate Change (IPCC) estimates indirect emissions from agricultural soils of the important greenhouse gas N_2O to be 0.75% the NO_3–N leached (IPCC, 2006). Indeed, it has been suggested that management practices to remove groundwater NO_3 through denitrification, such as riparian buffers, may be trading a water quality problem for an air quality problem (Groffman et al., 1998).

This laboratory study used small bioreactors to simulate a denitrification wall designed to intercept and treat subsurface groundwater. Our objectives were (i) to determine the rate of NO_3 removal by a wood chip bioreactor under a range of flow rates similar to those in tile drained fields in Iowa, (ii) to determine the partitioning of NO_3–N loss between denitrification and N immobilization and (iii) to quantify production of N_2O from a wood chip bioreactor.

Materials and Methods

Materials Description

Wood chips used in this study were the same as those used by Greenan et al. (2006), primarily oak (*Quercus* sp.), with an organic C content of 49.38% and total N content of 0.11%. The chips were 3 to 10 cm in length and approximately 0.5 to 1 cm on each side. The soil used to inoculate wood chips was oxidized glacial till (Eidem et al., 1999) taken 2 m below the surface of a Canisteo series soil (fine-loamy, mixed, superactive, calcareous, mesic Typic Endoaquoll) located in a corn/soybean-rotated field near Boone, IA (organic C content: 1.35%, total N content: 0.3%). Previous studies conducted with oxidized till used in this study and oxidized till from a nearby locations show that these subsoils support negligible denitrification rate without addition of C amendments (McCarty and Bremner, 1992; Cambardella et al., 1999; Greenan et al., 2006).

Bioreactor Setup

Bioreactors were constructed to simulate a cross-sectional area of a denitrification wall by using Schedule 40 polyvinyl chloride (PVC) pipe 50 cm long and 15.2 cm diam. Two acrylic plastic plates (15.2 cm diam. with random 3.2 mm diam. holes) were used to cap both ends of the column to diffuse the flow of water entering and leaving the columns. Silicone tubing samplers, similar to those described by Jacinthe et al. (1998), were constructed to collect N_2O dissolved in the effluent water. A coil of silicone tubing (1 m long, 3.18 mm inner diam. and 0.79 mm wall thickness (Cole Parmer Inst. Co., Vernon Hills, IL.) was inserted through the top end-cap above the perforated plexiglass plate. As effluent water flowed past the tubing, gases present in solution diffused into the tubing interior. Airspace in the tubing was sampled approximately every 2 d from Days 18 to 59 and analyzed for N_2O. Approximately 2635 ± 347 g (dry weight) of wood chips were mixed with 614 ± 1 g (DW) oxidized till and 1 L of distilled water until the till was evenly distributed on the wood chips. The mixture was packed into the bioreactors incrementally with tamping of the wood chips to ensure a tight matrix. After packing the column, the plexiglass diffuser plates were attached with silicone caulk and the end cap was attached using PVC cement to obtain an air and water tight seal.

Nitrate and Water Flow

The bioreactors were positioned vertically in a temperature and humidity controlled incubation chamber at 10°C (mean annual groundwater temperature in central Iowa) and 50% humidity (to avoid desiccation of collected sample). The experiment was designed to evaluate NO_3 removal at four different flow rates, with three replicate bioreactors at each flow rate (12 bioreactors total). The target flow rates were 2.9, 6.6, 8.7 and 13.6 cm d^{-1}; rates which bracket the rates observed in shallow groundwater flowing through oxidized till in central Iowa (Jaynes et al., 1999). Water from a 50-L carboy was pumped upward through the columns using peristaltic pumps and the effluent was collected in 1 L glass mason jars. No attempt was made to control concentration of dissolved oxygen (DO) in the water and it was assumed to be 12 mg L^{-1} (at 10°C). For the first 10 d, unamended distilled water was pumped at a rate of 6.6 cm d^{-1} to saturate the columns and to ensure proper calibration of the peristaltic pumps. From Days 10 to 14, flow rates were adjusted to achieve an intended target flow rates of 2.9, 6.6, 8.7 or 13.6 cm d^{-1}. The columns had a total volume of 9068 cm³ and an estimated average pore volume of 4844 ± 526 cm³ (53%), excluding interior pore volume of the wood chips. The size and shape of the wood chips is such that a network of large connected pores was created and we observed no

overt signs that water flow was limited by the hydraulic conductivity of the columns. Individual wood chips had an average density of 0.66 g cm^{-3}. The corresponding hydraulic retention time of water in the columns from the lowest to the highest flow rate was 9.8, 3.7, 2.8, and 2.1 d. From Days 15 to 54, distilled water amended with 50 mg L^{-1} NO$_3$–N (KNO$_3$) was pumped through the columns. Water samples were collected at 20 to 28 h intervals, acidified and stored at 4°C until they were analyzed for NO$_3$–N, NH$_4$–N, and dissolved organic carbon (DOC).

From Days 55 to 72, water was amended with isotopically enriched NO$_3$–N (10.00 atom % ^{15}N) to determine the amount of NO$_3$–N retained wood or in microbial biomass. The ^{15}N was added in the latter stage of the experiment to preclude effects from startup and focus on near-steady state conditions. From Days 73 to 85 unamended distilled water was pumped through the columns to flush out NO$_3$–N in the columns so that N analysis of the wood/till mixtures would not be affected by residual NO$_3$–N in the column. Flushing was deemed complete when NO$_3$–N was undetectable (<0.3 mg L^{-1}) in the effluent.

After flushing of NO$_3$–N from the columns was complete, the columns were disassembled. Samples of wood chip/till mixture from the top, middle, and bottom of the columns were removed for analysis of total C, organic C, total N and ^{15}N. The samples were prepared for analysis by drying at 70°C for 48 h, then ground in a Wiley Mill to <2 mm, and further ground to a fine powder using a Cyclone mill.

Nitrogen Mass Balance

Water samples were analyzed for NO$_3$–N (NO$_3$–N + NO$_2$–N) and NH$_4$–N on a Lachat autoanalyzer using the colorimetric reaction as described by Keeney and Nelson (1982). Total C, organic C, and total N content in the wood chips/till mixtures were determined by dry combustion using a Carlo Erba NA1500 NSC elemental analyzer. Samples were treated with acid before analysis to remove carbonate precipitates common to the till. For ^{15}N determination, solid samples were combusted in the elemental analyzer, which was connected to an isotope ratio mass spectrometer (Delta S, Finnigan MAT, Germany). Air samples were analyzed for N$_2$O using a Shimadzu gas chromatograph equipped with a ^{63}Ni electron capture detector (Parkin, 1985).

A mass balance approach was used to calculate the NO$_3$–N removal rates. At collection intervals the mass of NO$_3$–N in the effluent was subtracted from the mass of NO$_3$–N applied, resulting in the mass of NO$_3$–N removed. The mass of N removed was divided by the initial mass of wood chips in the bioreactor and the time (from previous sampling) to calculate the NO$_3$–N removal rate as mg NO$_3$–N removed g^{-1} wood d^{-1}. The removal rates were summarized by taking the mean of the three replicates of each flow rates when the flow rate treatment for each column were relatively constant (stable periods presented in Fig. 1). The ratio of NO$_3$–N removed to NO$_3$–N added was calculated in a similar way.

Ammonium losses in effluent began before nitrate was applied, so NH$_4$–N production was calculated by multiplying the concentration of NH$_4$–N in the effluent by the volume of water collected and summing these numbers over the 85 d of observations and expressed as the mg NH$_4$–N produced g^{-1} wood.

Nitrogen immobilized by the microbial community was calculated using the ^{15}N atom % present in the wood chips soil mixture using calculations from Mosier and Schimel (1993). The NO$_3$–N denitrified was calculated by subtracting the NO$_3$–N mass in the effluent water from the NO3-N applied (N removal) and correcting for NO$_3$–N immobilized.

Dissolved Organic Carbon Production

Dissolved organic carbon was determined using a Dohrmann DC-180 C analyzer. At the beginning of the dosing period, the concentrations of DOC declined in an exponential manner. The first two DOC concentrations were calculated based on the application of an exponential function to the 85 d data set. The DOC production through the 85 d of water dosing was calculated based on the concentrations of DOC measured in the effluent. Because fewer samples were analyzed for DOC, linear interpolation between observation points was used. The concentration of DOC was multiplied by the volume of water collected at the observation point and summed for the 85 d experiment. The mass of DOC produced was divided by the initial mass of wood chips in the bioreactor to yield mg DOC produced g^{-1} wood.

Nitrous Oxide Production

The N$_2$O concentrations in effluent water were calculated by assuming that the N$_2$O in the gas space of the silicone tubing was in equilibrium with the effluent water. This assumption is supported by the data of Jacinthe and Dick (1996) who reported that the time for N$_2$O to reach equilibrium when diffusing through silicone tubing with wall thickness of 2.4 mm was 4.4 h. We calculated aqueous phase N$_2$O concentrations using the universal gas law and the Bunsen coefficient for N$_2$O at 10°C (Tiedje, 1982). From the calculated aqueous N$_2$O concentrations and water flow rates, we calculated the mass of dissolved N$_2$O-N transported from the columns.

Statistical Analysis

Analysis of variance and Fisher's least significant difference (LSD) on NO$_3$–N removal rates were calculated using the general linear model provided in SAS (SAS Institute, 1985). Analysis of variance and LSD means separation on mass of initial wood chips added, NH$_4$–N and DOC leached in the experiment were determined using MINITAB.

Results

The NO$_3$–N concentrations in effluent water exiting wood chip bioreactors dosed with NO$_3$-amended water (50 mg NO$_3$–N L^{-1}) varied with flow rates (Fig. 1). Complete removal of NO$_3$–N was achieved at the mean flow rate of 2.9 cm d^{-1} throughout the Day 15 to 72 period, while average concentrations for mean flow rates of 6.6, 8.7 and 13.7 cm d^{-1} were 18.5, 24.2 and 35.3 mg NO$_3$–N L^{-1}, respectively. During the experiment, pump failure occurred in one or more of the columns causing average flow rates to decrease resulting in a decrease of NO$_3$–N concentration in effluent water. This effectively removed NO$_3$–N from the amended water and accounted for some of the deviation in mean concentrations, especially in the 8.7 cm d^{-1} flow rate columns. When the NO$_3$-amended water was
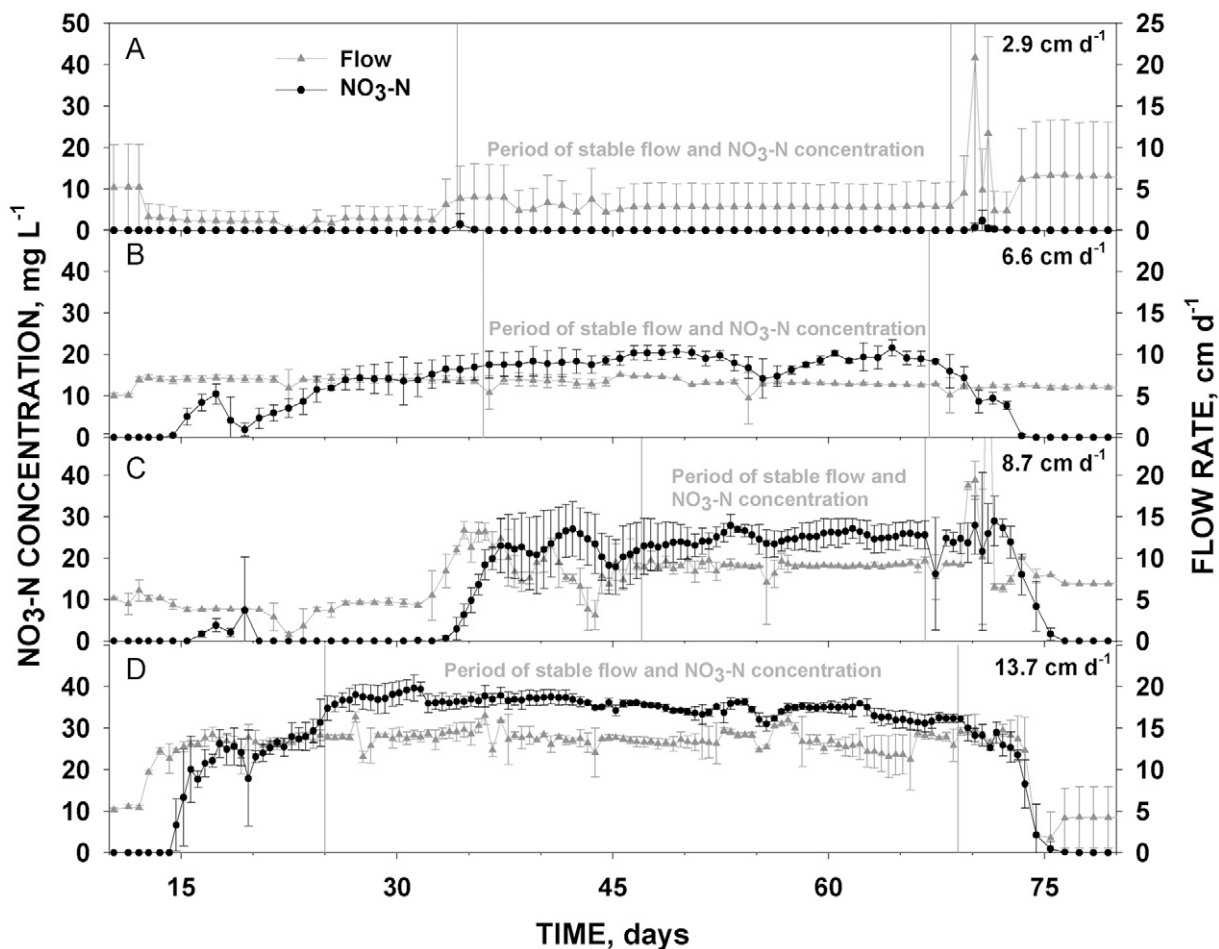
Fig. 1. Concentrations of NO_3–N in effluent water from four sets of bioreactors operated at average flow rates of (A) 2.9, (B) 6.6, (C) 8.7, and (D) 13.7 cm d^{-1}. Reactors were dosed with NO_3–N-amended water (50 mg L^{-1}) for 58 d. Nitrate-free water was applied in the 0 to 15 d period and 73 to 85 d period. Points and error bars are the mean and standard deviation of three replicate bioreactors. The nitrate removal efficiency was determined in the indicated periods of stable flow.

replaced with distilled water at Day 73, NO_3–N concentrations in effluent water declined over several days to negligible levels (detection limit: 0.3 mg NO_3–N L^{-1}).

Increased ($P < 0.001$) NO_3–N removal rates per kg of added wood occurred with increasing flow rates (Table 1). These removal rates were calculated during the periods when flow rates and effluent NO_3–N concentrations were generally stable (as indicated in Fig. 1). Lack of NO_3–N in effluent at the low flow rate indicates that NO_3–N was limiting and the removal rate for the low flow treatment was not included in the statistical analysis because the removal rate would be underestimated because of NO_3 limiting conditions. The N removal rate at the highest flow (13.6 cm d^{-1}) was only 38% greater than at the lowest flow (2.9 cm d^{-1}). The removal rates in Table 1 correspond to removal rates of 2.94, 4.15, 4.51, and 4.01 g N m^{-3} d^{-1} (bioreactor volume based) for the 2.9, 6.6, 8.7, and 13.6 cm d^{-1} flows, respectively.

The NO_3–N removal efficiency, calculated as a ratio of NO_3–N removed to NO_3–N added, decreased as the flow rate increased. Because of the dynamic changes in NO_3–N removal rates, based on flow rate the efficiency of removal could be described by the function ($y = 4.299x^{-1}$, $r^2 = 0.96$) where y is the ratio of NO_3–N removed to NO_3–N added and x is the flow rate in cm d^{-1} (Fig. 2).

Denitrification was the dominant mechanism of NO_3 removal in the wood chip bioreactors while NO_3–N immobilization was a minor process in all flow rate treatments (Table 1). We used $^{15}NO_3$–N to dose the reactors for 17 d and subsequently analyzed the wood chip/till substrate for ^{15}N to account for $^{15}NO_3$–N that could be immobilized as microbial biomass. Denitrification was calculated by subtracting NO_3–N in the effluent and NO_3–N immobilized from the NO_3–N that was added. There was a trend of increasing denitrification and decreasing N immobilization as the load of NO_3–N flowing into the bioreactors increased. There were significant differences in total mass of NO_3–N denitrified and N immobilized between the high and low flow rates. At the flow rate of 8.7 cm d^{-1}, the quantity of NO_3–N added and denitrified were reduced due to flows less than the target rate for these columns between Days 15 and 30 (Fig. 1). Less NO_3–N was added due to the flow rate averaging 4.6 cm d^{-1} for the first 31 d and no NO_3–N was observed in the effluent during this time. This can account for less NO_3–N denitrified compared to denitrification at the flow rate of 6.6 cm d^{-1}. At the flow rate of 2.9 cm d^{-1}, NO_3–N was not detected in the effluent. Therefore, NO_3–N may have been limiting and there was greater potential for total NO_3–N to be denitrified

Table 1. Nitrogen transformations and losses during 58 d of dosing with NO_3–N amended water. Data are the mean ± standard deviation for three replicate bioreactors at each flow rate.

Flow rate	Wood chips added	NO_3–N balance†			N_2O Produced	NO_3–N removal rate§	NH_4–N and DOC leached	
		Added	N not recovered	Immobilized‡				
cm d⁻¹	kg wood	——————mg NO_3–N kg⁻¹ wood chips——————			mg N_2O-N kg⁻¹ wood	mg NO_3–N kg⁻¹ wood d⁻¹	mg NH_4–N kg⁻¹ wood	mg DOC kg⁻¹ wood
2.9	2.39 ± 0.097 ab¶	580 ± 20 a	550 ± 20 a	40 ± 10 a	0.019 ± 0.007 a	11 ± 1#	44 ± 4 a	2230 ± 380 a
6.6	2.87 ± 0.439 bc	1320 ± 180 b	890 ± 130 bc	30 ± 5 ab	0.085 ± 0.037 a	13 ± 1 a	69 ± 6 b	2340 ± 270a
8.7	2.93 ± 0.070 c	1390 ± 50 b	820 ± 110 b	30 ± 10 ab	0.299 ± 0.210 a	14 ± 2 b	71 ± 11 b	2330 ± 150 a
13.6	2.34 ± 0.172 a	3080 ± 180 c	990 ± 50 c	20 ± 10 b	0.236 ± 0.195 a	15 ± 2 c	79 ± 8 b	3370 ± 210 b

† Nitrogen balance calculated from the entire 58 d period when the bioreactors were dosed with NO_3–N (Day 15 to Day 73).

‡ Nitrate-nitrogen immobilized was calculated from $^{15}NO_3$–N immobilized during the dosing period with $^{15}NO_3$–N and applied to the entire NO_3–N dosing period.

§ Nitrate-nitrogen removal rate calculated during dosing periods when flow rate and NO_3–N concentrations in effluent were generally stable.

¶ Values followed by different letters within each column are significantly different as determined by ANOVA and LSD ($P < 0.05$).

Flow rate of 2.9 cm d⁻¹ was not included in the statistical analysis because rate was underestimated due to lack of NO_3–N in the effluent.
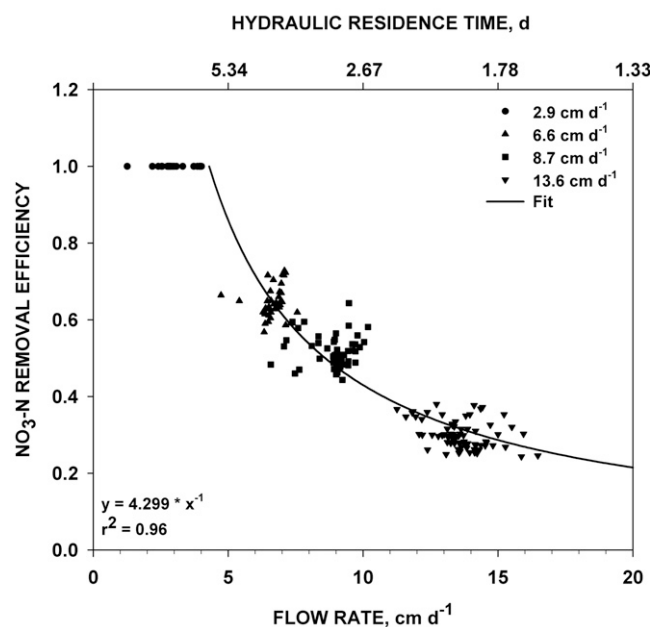

Fig. 2. Efficiency of NO_3–N removed (mg NO_3–N removed per mg⁻¹ NO_3–N added) in relation to flow rate in a wood chip bioreactor. The fitted line is a reciprocal equation ($y = a \times x^{-1}$) showing decreased efficiency of removal with increased water flow. Data points are the ratio of NO_3–N removed to NO_3–N added and are the daily mean of three replicate columns when NO_3–N concentration in the effluent and flow rates were relatively stable. Average flows are shown in the upper right. Corresponding hydraulic residence times are shown on the upper axis.

Nitrous oxide produced from Days 18 to 59 ranged from 0.019 mg N_2O-N kg⁻¹ wood chips to 0.299 mg N_2O-N kg⁻¹ wood chips (Table 1). While there was a trend of increased N_2O production with increased NO_3 inputs, this trend was not significant ($P > 0.176$). Averaged over all the flow rates, N_2O-N production accounted for 0.009% of the mass of NO_3–N that was added (range of 0.003–0.022% of the NO_3–N added). Similarly, N_2O production accounted for only a small fraction of the NO_3–N denitrified (ranging from 0.003–0.033% N_2O-N of the NO_3–N denitrified), indicating that the primary denitrification end product was N_2.

During the initial startup period, NH_4–N concentrations in the effluent ranged from 9 to 16 mg L⁻¹, while concentrations had

declined to 0.2 to 0.8 mg L⁻¹ at the end of 85 d of flow (Fig. 3). The quantity of NH_4–N leached after the initial startup phase seemed to be dependent on the volume of water that moved through the columns. Significantly more NH_4–N leached from the three greatest flow rate treatments than the low flow rate ($P < 0.05$) after 73 d of flow, including 15 d where no NO_3 was added. (Table 1). The leaching of NH_4–N from the columns seemed to occur independent of NO_3–N loading and appears to be unrelated to NO_3–N removal because the greatest quantity of NH_4–N occurred during the first 15 d of flow with distilled water. Greenan et al. (2006) conducted static incubations to assess NO_3 removal and found no production of NH_4–N by the same oxidized till (without wood) used in the present study. This also suggests that the wood was the primary source of this initial NH_4–N.

Dissolved organic carbon was also leached from the bioreactors, but there was not a clear relationship between DOC and initial mass of wood chips added. The range of DOC concentration in the effluent during the startup phase was 357 to 866 mg DOC L⁻¹ and declined to a range of 14 to 39 mg DOC L⁻¹ by the end of the experiment (data not shown). There was a positive trend between total mass of DOC leached and flow rate, but only the high flow rate (13.7 cm d⁻¹) was significantly greater than the other flow rates ($P < 0.05$) (Table 1). The origin of the DOC is likely to be wood, as the oxidized till contributes <1% of the total C in the bioreactors at the start of the experiment.

Discussion

Nitrate export from subsurface drained agricultural soils in the Midwest continues to impact surface water quality both by mass of N exported and concentration of NO_3–N. Farming practices to reduce load and concentration of NO_3–N have not been universally adopted and this has prompted research of alternative strategies. Bioreactors and denitrification walls built to promote denitrification of NO_3 contaminated water using tree bark, leaf compost, wood sawdust, and wood chips have been shown to effectively remove NO_3 and have reduced NO_3–N concentration in effluent water (Blowes et al., 1994; Robertson et al., 2000; Robertson and Cherry, 1995; Schipper and Vojvodic-Vukovic, 1998, 2000, 2001; Schipper et al., 2005; Van Driel et al., 2006). Similar results were obtained after installation of a wood chip denitrification wall in Iowa, where
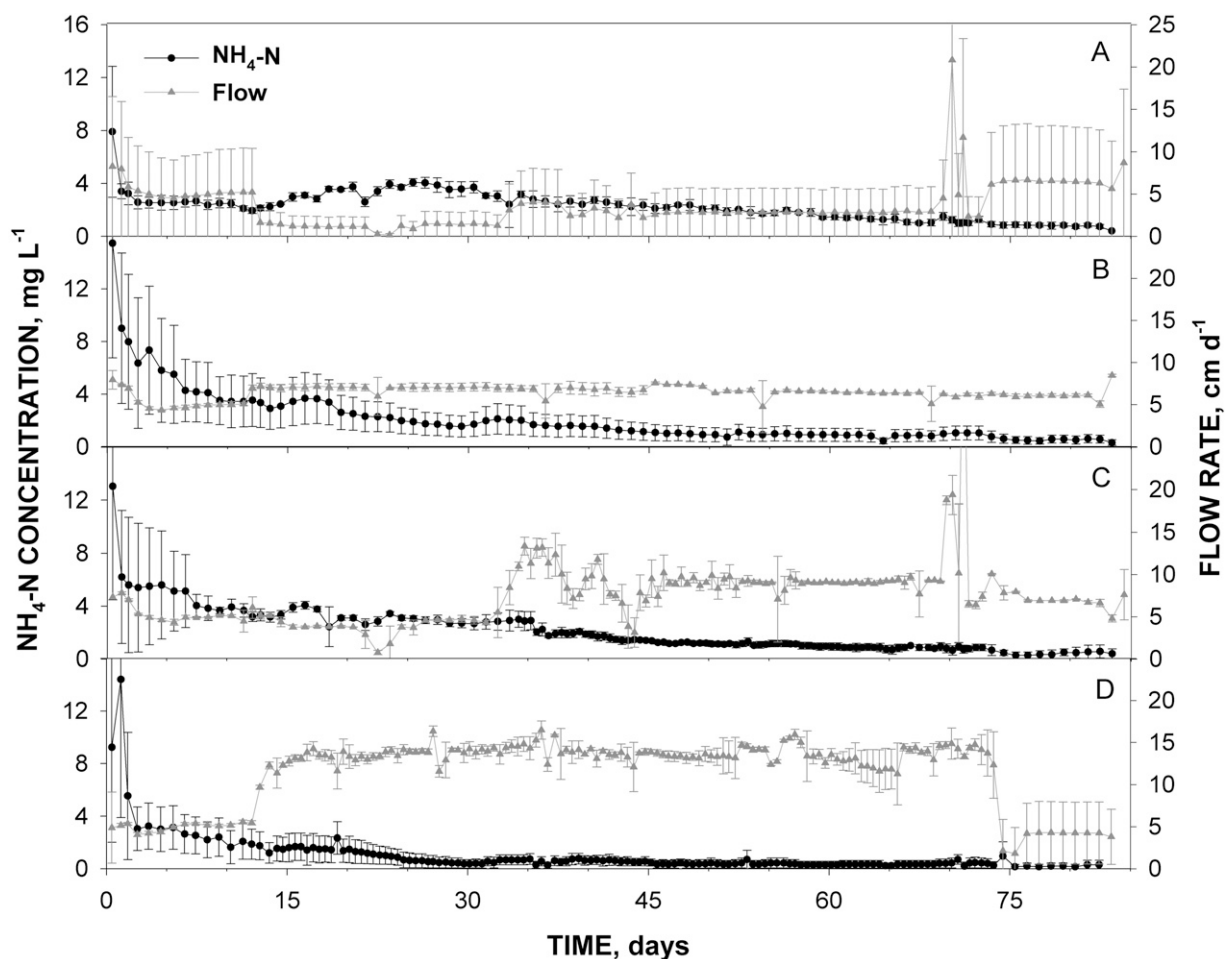
Fig. 3. Concentrations of NH_4–N in effluent water from four sets of bioreactors operated at average flow rates of (A) 2.9, (B) 6.6, (C) 8.7, and (D) 13.7 cm d^{-1}. Nitrate-free water was applied in the 0 to 15 d period and 73 to 85 d period. Points and error bars are the mean and standard deviation of three replicate bioreactors.

NO_3–N concentrations of subsurface drainage water were reduced to <10 mg NO_3–N L^{-1} in 4 of 6 yr (Jaynes et al., 2008).

We tested the effectiveness of wood chip bioreactors to remove NO_3–N at a starting concentration of 50 mg L^{-1} under a range of flow rates at temperatures that would be expected under field conditions. The efficiency of removal (i.e., NO_3–N removed vs. NO_3–N added ratio) decreased as flow rates increased. This may be partly due to the increased tranport of dissolved oxygen at the higher flow rates. Microbial metabolism removes this dissolved O_2 as it enters the reactor, but denitrification efficiency could be reduced as a consequence. Dissolved oxygen would also be entering denitrification bioreactors operating under field conditions. Mean efficiencies were 100, 64.3, 51.7, and 30.1% for the flow rates 2.9, 6.6, 8.7, and 13.6 cm d^{-1}. Complete removal would be expected up to a flow of 4.3 cm d^{-1}, which corresponds to a HRT of 8.1 d. Volokita et al. (1996) observed the same relationship when newspaper bioreactors were submitted to increasing flow rates. Increasing flow rates increase the daily load of N entering the bioreactor. While N removal efficiency declined with increasing flow and N loading, the N removal rates per unit mass of wood increased, but not proportionately to the increase in N. Our N removal rates expressed on a bioreactor volumetric basis (2.9 to 4.5 g N m^{-3} d^{-1})

are substantially greater than those from our wood chip bioreactor (0.6 g N m^{-3} d^{-1}) operating in the field (Jaynes et al., 2008), but similar to those reported by Robertson and Cherry (1995) of 3.2 to 6 g N m^{-3} d^{-1}, and Van Driel et al. (2006), 2.5 g N m^{-3} d^{-1}, but much less than those reported by Saliling et al. (2007). Saliling et al. (2007) used a methanol and mineral nutrient-amended solution to feed their bioreactors operated at ambient temperatures to obtain removal rates reaching 1300 g N m^{-3} d^{-1}.

Some evidence that denitrification is the mechanism responsible for NO_3–N removal has been presented previously, but the roles that N immobilization and loss of NO_3–N as N_2O had not been quantified. Schipper and Vojvodic-Vukovic (1998) reported a declining total N in their denitrification wall and suggested that N immobilization was not a substantial NO_3–N removal mechanism. Our use of ^{15}N to quantify the role of N immobilization yielded results similar to Greenan et al. (2006). Nitrate-nitrogen immobilization by the wood chip associated microorganisms was a minor mechanism of NO_3–N removal, accounting for 2.0 to 3.5% of NO_3–N removed. Dissimilatory NO_3 reduction to NH_4 (DNRA) is also a potential fate of NO_3 (Tiedje et al., 1982). Although not measured in this study, Greenan et al. (2006) observed undetectable rates of

DNRA (<10 mg N kg^{-1} wood chip) in 180 d anaerobic incubations of wood chips with added NO$_3$. After accounting for immobilized N we estimate that 96.5 to 98.0% of the N loss was due to denitrification.

Production of the greenhouse gas N$_2$O is a potential detrimental side effect associated with remediation of NO$_3$ in groundwater via biological denitrification. The Intergovernmental Panel on Climate Change recently published an emission factor (EF5-g) for computing indirect N$_2$O emissions based on the mass of NO$_3$ leached from agricultural lands (0.0075 kg N$_2$O-N/kg NO$_3$–N leached) (IPCC, 2006). This emission factor was developed primarily from observations of N$_2$O and NO$_3$ concentrations in groundwater. However, as Groffman et al. (2000) observed, "The conceptual basis for EF5--g is clearly flawed." This is primarily due to the fact that point-in-time N$_2$O/NO$_3$ concentration ratios do not reflect the dynamics of N$_2$O production/consumption in groundwater or the processes that ultimately impact the mass of N$_2$O released to the atmosphere. In our studies net N$_2$O production was measured directly. With this data and from the known NO$_3$ loading rates we calculate an average emissions factor (EF5-g) of 0.000097 g N$_2$O-N kg^{-1} N leached which is significantly less ($P < 0.05$) than the IPCC factor of 0.0075 g N$_2$O-N kg^{-1} N leached. A similar finding was reported by Clough et al. (2007) who observed N$_2$O fluxes from a subtropical stream was only 0.1% of the IPCC calculated flux. Of course it is possible that our measured N$_2$O production values could underestimate the total N$_2$O production if N$_2$ bubble formation and subsequent ebullition served to strip N$_2$O out of the liquid phase and bypass the silicone tubing samplers. The maximum bubble volume can be estimated using the procedure of Scardina and Edwards (2001). If it is assumed that all of the NO$_3$–N not recovered (Table 1) was converted to gas, then the estimated bubble volumes ranged from 1.1 to 2.1 L for the different columns. Using these values along with the mean N$_2$O concentrations measured in the silicone tubing samplers, we estimate that the maximum amount of N$_2$O lost in bubbles ranged from 0.005 to 0.08 mg N$_2$O-N which equate to 0.04 to 10.6% of the N$_2$O-N measured in solution. Thus, even with these additional potential unmeasured N$_2$O losses that could have occurred as a result of bubble ebullition the mass of N$_2$O produced averaged 0.010% of the mass of NO$_3$–N added, which equates to an emission factor of 0.000104.

Many factors influence the composition of N gasses resulting from biological denitrification. Increasing concentrations of NO$_3$, NO$_2$, O$_2$, and H$_2$S concentrations and decreasing pH and temperature generally result in increased N$_2$O/N$_2$ ratios (Firestone and Davidson, 1989). However, in an assessment of a conceptual model of N$_2$O production from soils Davidson et al. (2000) consider these factors and conclude that soil water content may be the single most important controller of N$_2$O emissions. Based on limited laboratory and field data, these investigators proposed a conceptual relationship between soil water filled pore space and net N gas (N$_2$ and N$_2$O) production. This model indicates that, at 100% water filled pore space, N$_2$O production from denitrification will be negligible relative to N$_2$ production (Davidson et al., 2000). Our results are consistent with the Davidson et al. (2000) hypothesis. The bioreactors in this study were water saturated; conditions conducive to the complete reduction of NO$_3$ to N$_2$.

Interest in denitrification walls and bioreactors is increasing, but variations in flow, NO$_3$ concentration and denitrification capacity contribute to uncertainty in design of denitrification systems. We created an empirical model that uses the relationship between measured NO$_3$–N removal rates and flow rates to predict concentrations of NO$_3$–N that could be treated to result in effluent concentrations of 10 mg NO$_3$–N L^{-1} (Fig. 4) under the conditions of this study. However, subsurface drainage is highly variable and seasonal. Peak discharge from field tiles can range from 200 to 1080 m^3 d^{-1} (Jaynes et al., 1999; Jaynes et al., 2001), but the majority of daily flows are <50 m^3 d^{-1}. Using the 1.37-ha area drained area described by Jaynes et al. (2001) as an example, and assuming 500 m^2 of effective wood chip filter surrounding the drain, a peak discharge event of 200 m^3 d^{-1} would produce an average flow through the wood chip wall of around 40 cm d^{-1}. Our model indicates that at this flow rate N removal from a 20 mg NO$_3$–N L^{-1} input concentration to 10 mg NO$_3$–N L^{-1} would not be possible. However, at the 50 m^3 d^{-1} discharge rate, this would be achievable. Using the relationship shown in Fig. 4, reductions from 20 to 10 mg L^{-1} of NO$_3$–N would be achievable up to flows of 16 cm d^{-1}, which corresponds to a HRT of 1.67 d.

Although this experiment was brief relative to the time an in situ bioreactor might be expected to be deployed, it provides quantitative data on the effectiveness of wood-derived bioreactors to stimulate denitrification of NO$_3$–N in water flowing at different flow rates. Nitrogen immobilization accounted for only a small portion of NO$_3$–N removal, thus it is concluded that denitrification was the primary fate of NO$_3$. Production of N$_2$O was on average 100 fold less than predicted by the IPCC emission factor for indirect N$_2$O emissions from NO$_3$ in groundwater. We would expect the N removal rates to decrease as the system ages and the wood decomposes. Long term (>8 yr) field studies are currently underway to assess the long-term efficacy of wood chip denitrification bioreactors.

Acknowledgments

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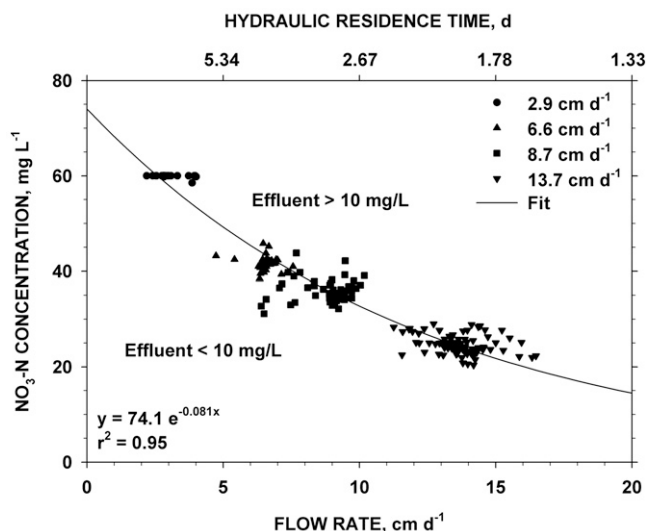
Fig. 4. Predicted NO$_3$–N concentrations entering a wood chip bioreactor resulting in an effluent concentration of 10 mg L^{-1} (fitted line) based on observed flow rates and NO$_3$–N removal rates. The equation for this is: [(mg NO$_3$–N removed g wood^{-1} d^{-1} × g wood) + (10 mg NO$_3$–N L^{-1} × flow rate)]/flow rate = input NO$_3$–N concentration. The 10 mg NO$_3$–N L^{-1} × flow rate represents the EPA MCL of 10 mg L^{-1} NO$_3$–N in effluent water. Flow rates have the units of cm d^{-1}. Combinations of flow rate and influent NO$_3$–N concentration falling below the line result in effluent concentrations less than the 10 mg L^{-1} target. Individual points represent mean daily NO$_3$–N removal rates during the stable flow periods shown in Fig. 1. Corresponding hydraulic residence times are shown on the upper axis.

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